Progress Report
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CARDIOPULMONARY TOXICITY INDUCED BY AMBIENT PARTICULATE MATTER (TRI CITY CONCENTRATED AMBIENT PARTICLE STUDY)

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DISCLAIMER

EXECUTIVE SUMMARY

The Tri City Concentrated Ambient Particle Study (Tri City CAPS) is designed to investigate the sources and components of fine particulate matter ($PM_{2.5}$) responsible for adverse health effects, with an emphasis on coal-fired power plant-derived PM. The Project is a multi-site field study to investigate the toxicity of secondary $PM_{2.5}$ derived from coal-fired power plants and other sources, including mobile sources. A portable ambient particle concentrator coupled with a mobile toxicological laboratory are employed to assess the health effects of CAPs in regions dominated by different PM sources. The Project includes three study locations, each to be evaluated during both winter and summer seasons to exploit different meteorological regimes. The first study location is located near the Ambassador Bridge in Detroit, MI, and is heavily influenced by both idling diesel truck traffic and gasoline-fueled commuter traffic. This site also corresponds to the location of an EPRI-funded air pollution epidemiology study. The second site is located in Steubenville, OH, an area dominated by both regional power plant-derived PM as well as local industrial sources. The third site is located in Maurice K. Goddard State Park in Northwest Pennsylvania, an area also heavily influenced by power plant emissions, but lacking urban or industrial influences. The selection of sites and seasons is based on achieving the highest degree of variability in PM composition and contribution from different sources. Spontaneously hypertensive (SH) and normal (Wistar-Kyoto) rats are exposed to CAPs from these locations for 13 consecutive days and assessed for a wide suite of cardiopulmonary endpoints. The rats are implanted with telemeters and evaluated for pulmonary, systemic, and cardiovascular effects. At the same time, comprehensive exposure characterization is carried out to enable linking of adverse health impacts with PM composition. Also, importantly, source apportionment is carried out to enable attribution of toxicological effects to specific PM sources.

This report documents progress made on the Project during the period of October 1, 2005 through December 31, 2005. During this reporting period, laboratory chemical analyses were conducted on CAPs and $PM_{2.5}$ samples collected during the first round of fieldwork (Detroit, July 16-28; Site 1, Season 1). In addition, assays were performed on collected biological samples from this round of fieldwork, and processing of electrocardiogram (ECG) data was performed.

Exposure parameters measured included acidity; sulfate, nitrate and ammonium ion; elemental carbon (EC); organic carbon (OC); and trace elements (total and water soluble). A novel semicontinuous slurry sampler was used to obtain finer time resolution for trace metals. Ambient criteria gases (CO, SO_2, NO_X, and ozone) and meteorological parameters were also measured. Toxicological endpoints evaluated included: ECG analysis on downloaded data from telemetered animals, tissue histological analysis (heart and airways), bronchoalveolar lavage (BAL) fluid analysis for cellularity and soluble markers of inflammation and injury, blood analysis for a complete blood count, and plasma analysis for soluble markers of inflammation and cardiac injury.

Most of the characterization analyses on the CAPs and ambient $PM_{2.5}$ samples have been completed, with the exception of the metals analyses (slurry samples and ambient $PM_{2.5}$). Concentration enrichment factors ranged from 6 to 65 during the exposure period; variations are believed to be due to environmental factors such as ambient PM levels/size distribution, and meteorological conditions such as humidity. CAPs concentrations ranged from 81 to 1638 μg/m, with a mean exposure concentration of 519 ± 505 $\mu g/m^3$. CAPs mass concentrations were dominated by organic carbon, elemental carbon, sulfate, nitrate, and ammonium. On average, the composition of ambient $PM_{2.5}$ and CAPs was similar. Elemental carbon was not concentrated as well as the other major PM constituents, probably due to the fact that EC is primarily found in the ultrafine fraction.

Toxicological analyses are underway. Histologic processing of pulmonary tissues is expected to be completed by mid-March 2006. Morphometric analysis to determine mucosubstances in the airways has been completed; results indicate that CAPs exposure increased the volume densities of mucosubstances in WKY rats, suggesting that CAPs may induce an increase in the production of airway mucous and possibly an increase in mucous-producing cells. Biochemical analyses on collected bronchoalveolar lavage fluid are underway; results are expected to be reported in the next progress report. Electrocardiogram data have been subjected to an initial, automated analysis to determine parameters of heart rate variability; more detailed analyses, including normalization using baseline data and comparison between exposure days and treatments, are underway.

Source apportionment analyses and statistical analyses linking PM composition to toxicological effects will be reported at least in part in the next progress report. Fieldwork for Site 1, Season 2 (Detroit, winter) is scheduled for February 11 – 23, 2006.

PROJECT PROGRESS

Approach

The monitoring location for the work described in this report is Maybury Elementary School, located at 4410 Porter St. in Southwest Detroit. This site is located ½ mile from the Ambassador Bridge, the busiest border crossing between Canada and the United States with daily traffic volume near this site estimated to be as high as 100,000 vehicles/day. Major contributors to the local traffic congestion are diesel trucks involved in import-export trade, and with materials delivery to and from auto and manufacturing industries in Detroit. Thus, PM at the site is heavily dominated by diesel emission-derived particles. The site is also located 1 block from Interstate 75, the primary southbound route from Detroit to Ohio, thereby contributing gasoline emission-derived PM to exposures conducted at this location.

The Ambassador Bridge area is also being studied as part of the Detroit Exposure and Aerosol Research Study (DEARS), a large EPA-funded exposure assessment study to investigate the importance of different PM sources to residential and personal exposures. DEARS specifically targets the Ambassador Bridge site as being a location heavily impacted by mobile sources. Coupled with DEARS is the EPRI-funded Detroit Cardiovascular Health Study, an epidemiological panel study to be conducted in the same cohort as the exposure study, and at the same time as the proposed Project. Thus, the Project will integrate the toxicology, epidemiology and exposure assessment disciplines for the same site.

The specific approach employed at Site 1, Season 1 consisted of several discrete tasks, briefly described below (additional detail can be found in the first progress report dated October 31, 2005).

Mobile Concentrator/Laboratory Setup: A mobile concentrator/laboratory (“AirCARE1”) was used to generate the CAPs for animal exposures and serve as the location of the toxicological assessments. Prior to transportation of AirCARE 1 to Detroit, a performance check of the concentrator and exposure chambers was conducted at Michigan State University (MSU). All sampling equipment was set up, tested, calibrated, and operational several days before the animal exposures began. Data acquisition systems were synchronized and final preparation of the concentrator and sampling systems were verified prior to each exposure day. Electrocardiogram (ECG) monitoring hardware was installed, and animals were moved into the laboratory 2 days prior to exposures to acclimatize to the laboratory environment

Particle Concentration and Animal Exposures: Exposure aerosol was extracted from ambient air outside the mobile lab using a Harvard/EPA fine ambient air particulate concentrator (Sioutas et al., 1995). *In vivo* inhalation toxicology experiments were carried out using 11-12 week old spontaneously hypertensive (SH) rats (compromised) and Wistar-Kyoto (WKY) rats (normal). Two to three weeks before exposures, rats were surgically implanted with telemetry transmitters for continuous ECG recording. Exposures took place in two stainless steel Hinners-type whole body inhalation chambers, and were 8 hours in duration for 13 consecutive days. Continuous ECG readings were recorded before, during and after exposures. Rats were returned to their individual cages overnight.

Exposure Characterization: Concurrent with the animal inhalation exposures, intensive characterization of the CAPs was conducted using state-of-the-art monitoring methods. Analysis of CAPs was conducted for both organic and inorganic constituents, including mass, acidity, sulfate, nitrate and ammonium ion, elemental carbon (EC), organic carbon (OC), and trace elements (total and water soluble). A novel semicontinuous slurry sampler was used to obtain finer time resolution data for trace metals and EC/OC. Ambient criteria gases (CO, SO_2, NO_X, and ozone) and meteorological parameters were also measured.

Toxicological Assessments: Extensive toxicological evaluation was conducted, focusing on cardiopulmonary endpoints. Animals were sacrificed 24 hours after the last exposure for collection of tissues for biochemical, molecular, and pathological analyses.

ECG analysis for Site 1, Season 1 data is underway, through subjective ECG waveform review, automated ECG analysis, and calculation of heart rate variability, including time-domain measures of SDNN (standard deviation of the normal-to-normal intervals), RMSSD (square root of the mean squared differences of successive normal-to-normal intervals), and SDANN (standard deviation of the average normal-to-normal intervals calculated over short periods).

Tissue histological analysis was carried out on heart and airway tissues from lung and nose to identify exposure-related alterations in cardiac and pulmonary tissues.

Bronchoalveolar lavage fluid (BALF) was analyzed for cellularity and soluble markers of inflammation and injury (TNF-α).

Blood analysis was performed for a complete blood count (CBC).

Plasma analysis was performed for soluble markers of inflammation and cardiac injury (cardiac troponin, C-reactive protein, myeloperoxidase, and asymmetric dimethylarginine [ADMA]).

Data Analyses: The complete data analyses for Site 1, Season 1 have not yet been initiated; however, this section describes the approach that will be employed for such analyses.

Source apportionment analyses will be conducted on the exposure CAPs in order to enable linking of specific PM sources to biological effects. This work will be carried out using multivariate receptor modeling techniques, including Positive Matrix Factorization (PMF) and the UNMIX model. HYSPLIT4 (Hybrid Single-Particle Lagrangian Integrated Trajectory) Model will be used to calculate backward air mass trajectories from each of the exposure sites during each study period to allow the investigation of sources and the source areas impacting the site. Specific chemical tracers that will be examined and used in the source attribution analyses include S, Se, K, Ca, and Fe (coal), Fe, Zn, Mn, Cu, Pb, EC, and OC (motor vehicles), and Sb, Pb, Zn, and Hg (waste incinerators).

Toxicological data will be expressed as the mean group value ± the standard error of the mean (n = 8/group). Analyses of variance (ANOVA) will be performed using the factors of exposure (air vs. CAPs) and strain (SH vs. WKY) for each field site. Comparisons across sites (Detroit, Steubenville, and Goddard Park) within treatment groups will be made by ANOVA using factors of exposure, strain and site. Correlations of physicochemical characteristics of CAPs with toxicological endpoints will be made by Pearson Product Moment analysis within each strain, using CAPs mean values for entire 13-day exposures at each site. Correlations of CAPs physicochemical characteristics with ECG endpoints will compare mean 30-minute data points within rat strains. A time series analysis will be performed to detect potential lag effects of CAPs exposure metrics with ECG abnormalities using SPSS 10.0 statistical software. Upon completion of this analysis, the source contributions calculated from the 30-minute averaged gaseous and PM composition data will be merged with the ECG data to investigate specific source impacts..

Objectives

The primary objective of the Project is to evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) coal-fired power plant and traffic-related PM. Secondary objectives of the study are to (1) provide insight into toxicological mechanisms of PM-induced cardiopulmonary effects, particularly as they relate to susceptible subpopulations; and (2) generate toxicological data to directly correspond to epidemiology and exposure assessment data from concurrent studies being conducted at one of the Project locations, providing a rich dataset of human and animal data exploring the associations between PM sources and components and health.

Results and Discussion

Exposure Results

Extensive chemical and elemental analyses have been completed on the ambient and CAPs samples. These include organic (OC) and elemental carbon (EC) by thermal optical reflectance carbon analyses, acid gases and aerosols and major ions including sulfate, nitrate, and ammonium by ion chromatography, as well as a suite of trace elements by inductively coupled mass spectrometry (ICP-MS) performed in a Class 100 laboratory.

Tables 1 and 2 detail the measurements of ambient PM and CAPs that were performed in Detroit in July 2005, and all the analyses that have been conducted or that are in progress. CAPs samples were collected during 8-hour animal inhalation exposures (7:00 am – 3:00 pm). The CAPs mass was determined by placing 47-mm Teflon filters (Gelman Science) on the back of the animal exposure chamber at flow rate of 2 L/min. In addition, pre-baked Quartz filters (Gelman Science) were placed in filter packs mounted on the animal exposure chamber and sampled at a flow rate of 3 L/min. A Micro-Orifice Impactor (MOI) (MSP Corporation) was placed after the concentrator’s second stage to determine the size distribution and chemical characteristics of CAPs.

A Rupprecht and Patashnick Series 1400a TEOM was placed in-line after the third stage to continuously measure the concentrated fine mass concentration during the exposure periods. An identical TEOM was operated continuously outdoors to provide 30-minute $PM_{2.5}$ concentrations for the entire sampling campaign. To characterize the ambient PM size distributions, and to collect size-segregated samples for subsequent chemical and elemental analysis, the multi-stage MOI and $PM_{2.5}$ cyclone samples were collected. Gaseous air pollutants were measured continuously, including ozone (O_3), sulfur dioxide (SO_2), nitric oxides (NOx), and carbon monoxide (CO). Meteorological parameters including temperature, relative humidity, precipitation, wind speed and direction were measured to assess the day-to-day variability in local transport pathways and emission source influences.

Table 1. Status of ambient PM measurements and analyses

Measurement	PM Property	Sampling Medium	Sample Duration (hr)	Analytical Method	Progress
TEOM	Mass	-	Continuous	-	-
SMPS 3936	Size (0.02-1 μm)	-	Continuous	-	-
SEAS	Trace elements	MQ	Every 30-min	ICP-MS	In progress
MOI	Size (10 stages)/trace elements	Teflon	24	Gravimetric	Completed
Filter ($PM_{2.5}$)	Trace elements	Teflon	8	ICP-MS	Completed
ADS ($PM_{2.5}$)	Acid gases & aerosols and major ions	Teflon /denuders	8	IC/pH	Completed
Filter ($PM_{2.5}$)	Elemental & organic carbon	Quartz	8	TOA	Completed

Table 2. Status of CAPs measurements and analyses

Measurement	PM Property	Sampling Medium	Sample Duration (hr)	Analytical Method	Progress
TEOM	Mass	-	Continuous	-	-
Aethalometer	Black carbon	-	Continuous	-	-
APS 3021	Size (0.5-20 μm)	-	Continuous	-	-
MOI (stage 2)	Size (8 stages) /trace metals	Teflon	8	Gravimetric ICP-MS	ICP in progress
Filter	Trace elements	Teflon	8	Gravimetric ICP-MS	Completed
Filter	Acid aerosols and ions	Teflon/ denuders	8	Gravimetric IC	Completed
Filter	Elemental & organic carbon	Quartz	8	TOA	Completed

A. Gravimetric analysis and determination of concentration enrichment factors (CEFs)

The concentrations of ambient $PM_{2.5}$ and CAPs in the animal chamber were used to calculate concentration enrichment factors (CEFs); ratios of concentrated particle mass concentration to ambient concentration were calculated for each inhalation exposure period to evaluate concentrator performance for each inhalation exposure period (Table 3). The average CEF for the intensive campaign was approximately 25. As shown, daily ambient $PM_{2.5}$ mass concentrations during the exposure periods in July ranged from 8 to 44 μg/m^3. The average CAPs concentration during the 13-day exposure period was 519 ± 505 μg/m^3.

The Harvard group previously characterized the concentrator and found that CEFs were likely to be influenced by environmental factors such as levels of ambient PM concentration and size distribution, and meteorological conditions such as humidity. More specifically, the CEF is likely to be influenced by any ambient condition that influences the ambient particle size distribution. The fraction of the ambient mass near and below the cutpoint (0.1 μm) has an effect on the overall CEF. When the mass median aerodynamic diameter of the ambient particle mass size distribution is smaller than the cutpoint, a large portion of the ambient particle mass is likely to be in a size range that is concentrated less efficiently, resulting in a lower CEF. Thus, high RH and high PM levels tend to increase the CEFs. The Harvard group also observed that the operation of the concentrator, including the minor 3 pressure drop, the minor-to-total-flow ratio, and the alignment of the slits of the virtual impactors had a significant impact on the CEFs observed.

The present study was not was not supportive of the Harvard findings of meteorological conditions and ambient concentrations influencing CEFs; due to personnel changes during this field campaign, precise alignment of the slits of the virtual impactors was not achieved for optimal operation of the concentrator (CEF~30) for the first several days. These issues have been addressed and the details will be discussed in the final report.

Table 3. Average ambient $PM_{2.5}$ and CAPs concentrations during 8-hour exposure period, Detroit, Summer 2005 (Site 1, Season 1).

	CEFs	CAPs (μg/m^3)	Ambient (μg/m^3)	Notes
7/16/05	14	575.6	42.2	heavy rain event recorded
7/17/05	65	1437.4	22.3	concentrator clogged several times
7/18/05	37	1638.2	44.4	
7/19/05	27	360.1	13.5	
7/20/05	12	254.7	21.4	
7/21/05	38	594.5	15.7	
7/22/05	23	313.8	13.6	
7/23/05	13	115.5	8.7	
7/24/05	7	81.0	11.6	rain event in AM recorded
7/25/05	6	82.5	13.9	
7/26/05	30	843.1	28.0	
7/27/05	27	213.3	7.8	
7/28/05	24	239.5	10.1	
Average± std	**25±16**	**519±505**	**19±12**	

CEFs: concentration enrichment factors

B. Chemical characterization of ambient PM and CAPs

As expected, both CAPs and ambient $PM_{2.5}$ mass concentrations during these campaigns were dominated by organic carbon, elemental carbon, sulfate, nitrate and ammonium. Figure 1 shows daily variations of major chemical composition in the exposure chamber during each 8-hour exposure period. The concentration of particulate organics was estimated by multiplying the measured concentration of organic carbon by a factor of 1.8 as suggested by Turpin and Lim (2001). The crustal element component was estimated from concentrations of Fe, Al, Si, and Ca. Silica was estimated as potassium (K)/0.15 (Keeler, 1987) and an estimation of oxygen content of these crustal elements was applied and calculated as 2.14Al+2.43Fe+1.54Si.

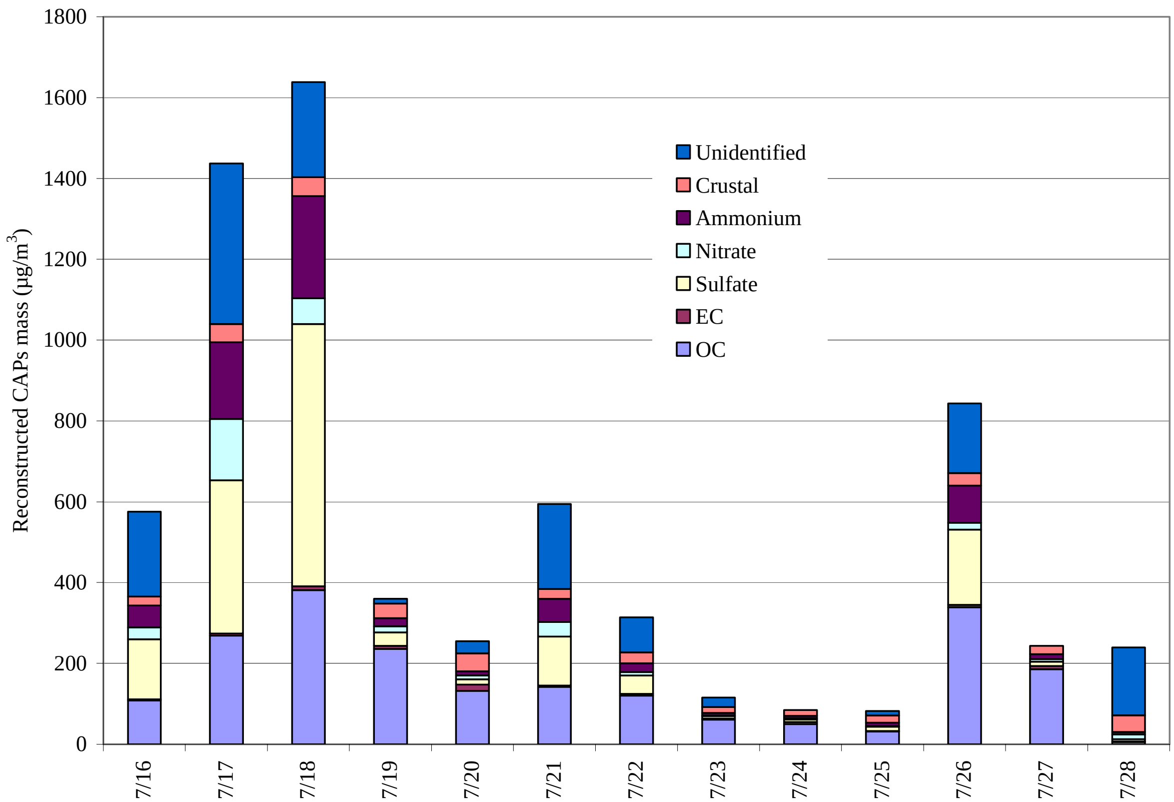

Figure 1. Daily variations of major chemical composition in CAPs during 8-hr exposure period.

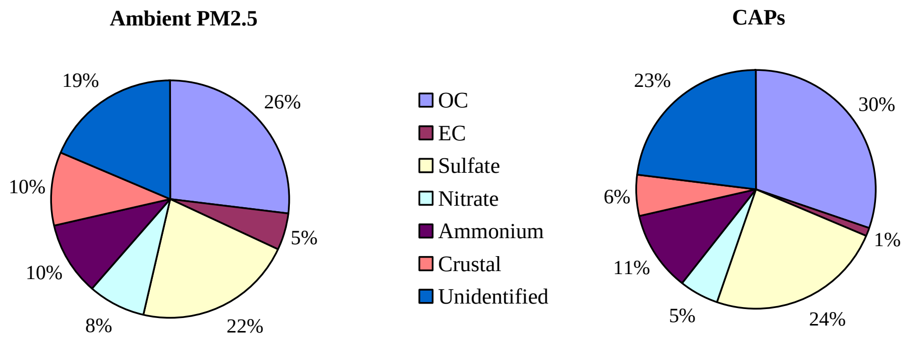

Figure 2. Average major chemical composition of ambient $PM_{2.5}$ and CAPs during the 13-day exposure period in July 2005 (preliminary results).

Figure 2 shows preliminary results of the major constituents of the ambient $PM_{2.5}$ and CAPs measured during the 13-day inhalation exposure periods in July 2005. Although some variability was observed due to some fluctuations of day-to-day concentrator performance and meteorological conditions, the overall average composition of the major constituents of the CAPs reflected that of ambient particles. However, elemental carbon (EC) was not concentrated as well as other major constituents. It was speculated that EC, which was dominated by ultrafine particles, was not concentrated since the fine particle concentrator increases the concentration of particles in size range 0.1-2.5 μm most effectively, and EC particles just passed through the concentrator.

In both Figures 1 and 2, we observed a high percentage of unidentified PM. Most of this fraction can be explained by aerosol associated water. Previous study has shown that PM water content was strongly dependent on deliquescent points of chemical composition and concluded that liquid water could represent a significant mass fraction of aerosol concentration at a relative humidity above 60% (Pilinis et al. 1989). The relative humidity of the exposure chamber during July often exceeded 60% and major portion of the unexplained fraction of the total mass may be explained by particle-bound water. This will be discussed in the final report.

C. Physical characterization of ambient PM and CAPs

Table 4 shows the number concentration of 0.5 – 2.5 μm particles in the chamber during the exposure period. As shown, the average concentration to which animals were exposed varied significantly on a daily basis, ranging from 65 – 1494 cm^{-3}. Figure 3 is a typical number size distribution of 0.5 – 20 μm particles in the chamber during an exposure period. As expected, the number concentration decreases monotonically with increasing particle size.

Table 4. Number concentration of 0.5 - 2.5 μm particles in the animal chamber during the exposure period.

Date	n	Mean	Median	Min	Max	SD
07/16/05	96	723	809	146	1478	349
07/17/05	95	1293	1112	113	2623	690
07/18/05	96	1483	1697	255	2377	596
07/19/05	93	220	156	85	694	153
07/20/05	96	128	101	59	265	56
07/21/05	95	549	543	300	872	114
07/22/05	91	175	176	112	265	46
07/23/05	98	83	73	50	161	26
07/24/05	94	65	60	2	178	61
07/25/05	96	65	4	0	253	87
07/26/05	97	560	627	19	1150	349
07/27/05	95	162	171	91	247	41
07/28/05	93	153	103	40	505	118

Concentration unit: cm^{-3}; n: sample size; SD: standard deviation.

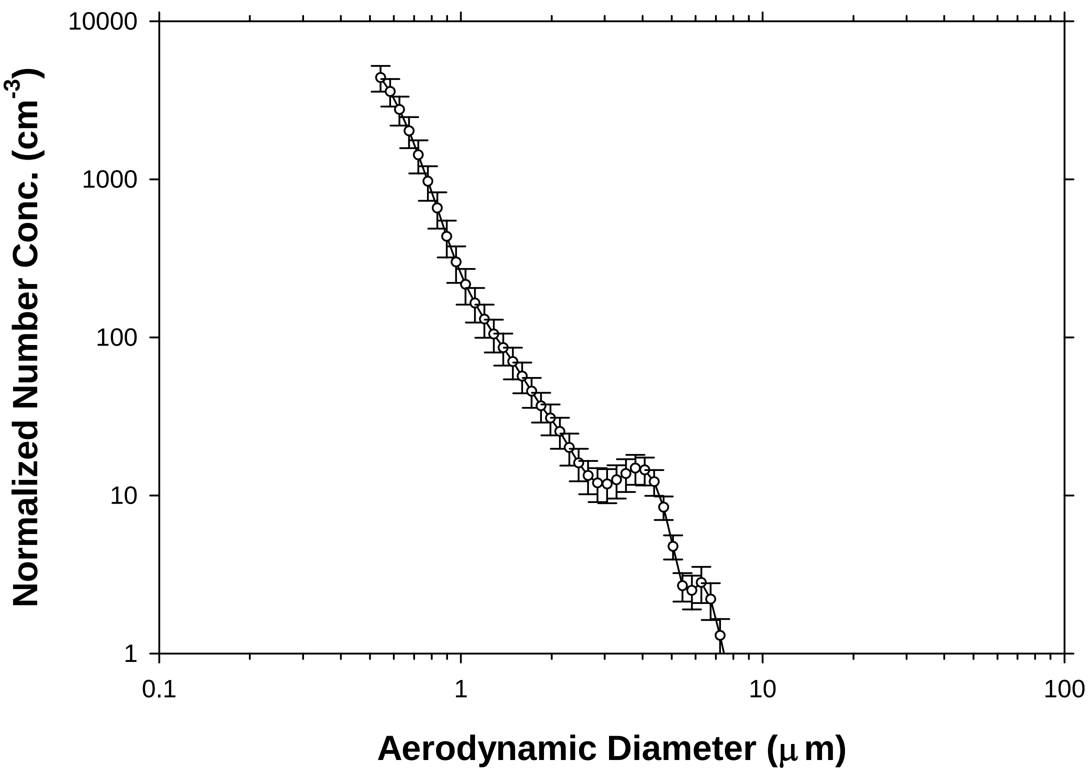

Figure 3. Typical average number-size distribution of 0.5 – 20 μm particles in the chamber during the exposure period. The error bar represents one standard deviation around the mean

Ultrafine particle mass concentrations, gas concentrations, and meteorological conditions were provided in the previous progress report dated October 31, 2005.

Toxicological Results:

A. Lung tissue for microscopic analysis

Since the last progress report, we have completed the histologic processing of all the pulmonary tissues taken from the 32 rats that were necropsied after the end of the 13 day-exposure period. Following the project protocol, the intrapulmonary airways of the fixed left lung lobe from each rodent were microdissected. Beginning at the lobar bronchus, airways were split down the long axis of the largest daughter branches (i.e., main axial airway; large diameter conducting airway) through the twelfth airway generation. Tissue blocks that transverse the entire lung lobe at the level of the fifth and eleventh airway generation of the main axial airway were excised and further processed for routine light microscopic examination. These tissue blocks were embedded in paraffin, sectioned at a thickness of 5 microns and stained with hematoxylin and eosin for routine histopathologic examination and with Alcian Blue (pH 2.5)/Periodic Acid Schiff (AB/PAS) stain for mucosubstances in the surface epithelium lining the conducting airways. Microscopic examination of these tissues is currently being conducted by the study pathologist, Dr. Harkema, to identify any CAPs-induced histopathology. Potential lesions in lungs from animals exposed to CAPs include inflammatory cell infiltrates, epithelial cell injury, proliferation and remodeling (mucous cell metaplasia), alveolar edema and peribronchiolar or alveolar septal fibrosis. We estimate that Dr. Harkema will finish his histopathologic examination of these tissues by mid-March, 2006.

Morphometric analysis to determine the amount (volume density) of AB/PAS-stained mucosubstances in the respiratory epithelium lining the proximal (generation 5) and distal (generation 11) airways in the left lung lobe of each rat was completed. We found that WKR rats had greater volume densities of intraepithelial mucosubstances in both the proximal and distal airways compared to those in SH rats. Interestingly, CAPs exposure significantly increased the volume densities of intraepithelial mucosubstances in only the airways of WKY rats. These data suggest that CAPs exposure induced an increase in the production of airway mucus and possibly an increase in the number of mucus-secreting cells in the airways of the WKY rats, but not in the SH rats.

B. Analyses of Bronchoalveolar Lavage (BAL) Fluid.

Results of the BAL analysis were presented in preliminary form in the last progress report. After statistical analysis of the data, we found that CAPs exposure did not cause any significant cellular changes in the BAL fluids that would indicate a CAPs-induced inflammatory response in the lungs of any of the exposed rats. The number of neutrophils in the BAL fluid of all the rats were extremely low indicating no acute pulmonary inflammation. We did find that all of the WKY rats, irrespective of the type of exposure, had a greater number of total cells in their BAL fluid compared to SH rats. This was due to more alveolar macrophages in the BAL fluid of WKY rats compared to SH rats.

The supernatant of the collected BAL fluid from each rat was stored at -80°C at the time of collection for future analysis of protein, and for a ELISA analysis to detect rat TNF-α. We expect that these biochemical analyses will be completed before the next progress report.

C. Electrocardiograms and Telemetry

Heart rate data collected during for Site 1, Season 1 has been subjected to an initial, automated analysis to determine parameters of heart rate variability, including time-domain measures of IBI (interbeat interval), SDNN (standard deviation of the normal-to-normal intervals), RMSSD (square root of the mean squared differences of successive normal-to-normal intervals), and SDANN (standard deviation of the average normal-to-normal intervals calculated over short periods). Recordings were taken for 10-second durations every 5 minutes during the 8-hour exposure period, as well as through the evening hours after the rats had been returned to their cages. Furthermore, extended baseline recordings were taken over a 24-hour period in animal facilities at Michigan State University.

Inter-beat interval data were extracted from raw data recordings using commercial analysis software (Dataquest ART Version 3.2, DSI-Transoma,) and placed into tabulated form for subsequent analysis using SAS. To eliminate artifacts and signal noise, data were clipped by removing the top and bottom 5% of the data values. Standard deviation (SAS) and rMSSD (Excel formula) were determined from the clipped data. To date, a cursory analysis of the data collected during the 8-hour exposure periods has been completed, and heart rate and SDNN data are presented in Figures 4 and 5. Note that data for July 21 are not included in these figures as the concentrator was not operating correctly during portions of the day. These raw data have yet to be normalized with regard to baseline data, and comparisons between exposure days and treatments have not been made. These analyses are underway, and future efforts will be to study the waveform morphology, rhythm analysis, QT and QTc, and power spectrum analysis.

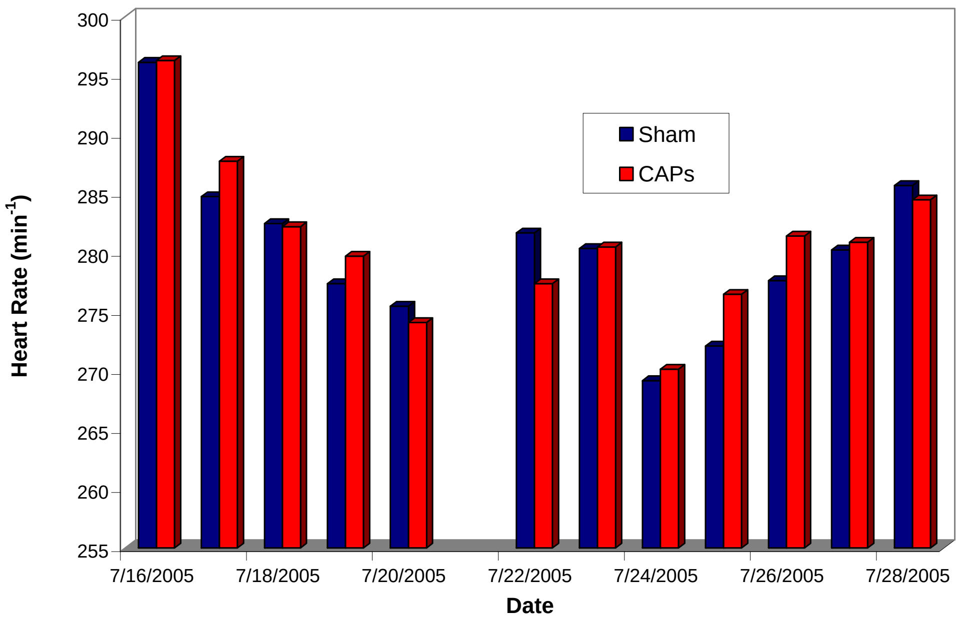

Figure 4. Mean daily heart rate in CAPs-exposed and sham rats, Detroit, Summer 2005.

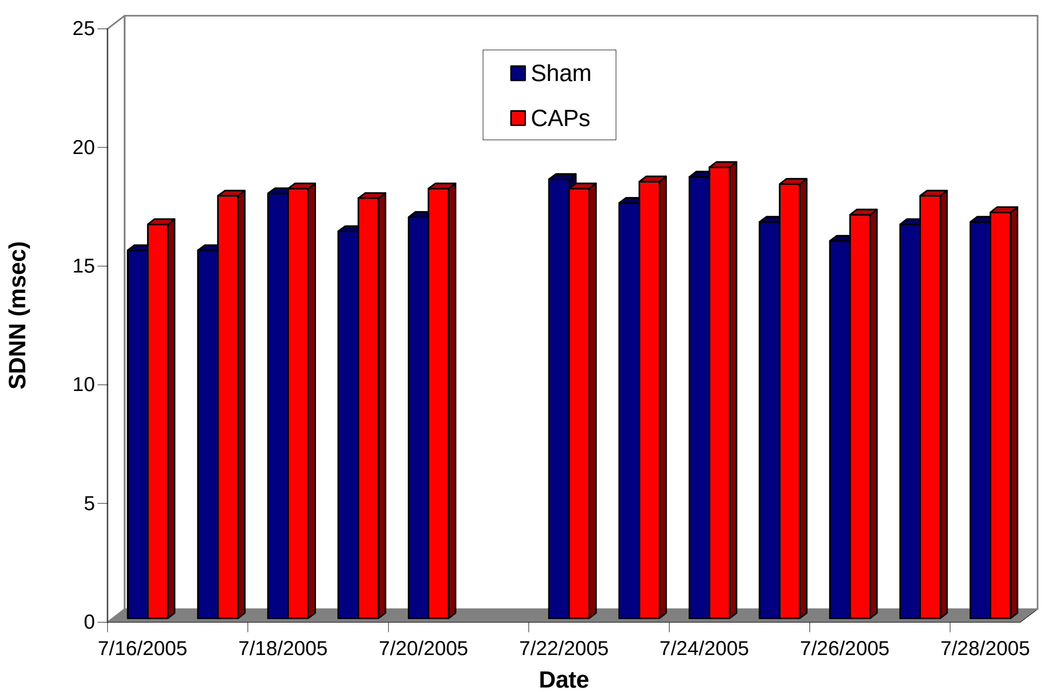

Figure 5. Mean daily SDNN in CAPs-exposed and sham rats, Detroit, Summer 2005

Conclusions

We have successfully completed the first field sampling campaign for the Project. Remaining exposure and toxicological analyses are underway and expanded results will be reported in the next progress report (April 30, 2006). Fieldwork for Site 1, Season 2 is scheduled for February 11 – 23, 2006. Fieldwork in Steubenville (Site 2, Season 1) will take place in Summer 2006.

COST STATUS

The table below summarizes the budget and expenditures to date. These costs include subcontractor costs (University of Michigan, Michigan State University).

Total federal funds authorized for this funding period	$312,000
Total outlays	$58,838
Recipient share of outlays	$16,089
Federal share of outlays	$42,749

SCHEDULE STATUS

The project is on schedule. We anticipate no problems in meeting the next milestone, Completion of Field Experiments at Site 1, by 6/30/06. Overall progress on the Project tasks is shown in the Table below.

Technical Progress -- 6 months

Task #	Description	Planned % completed	Actual % completed
1	Field Experiments at Site 1, Season 1	100%	90%
2	Field Experiments at Site 1, Season 2	0%	0%
3	Data Analysis for Site 1	14%	10%
4	Field Experiments at Site 2, Season 1	0%	0%
5	Field Experiments at Site 2, Season 2	0%	0%
6	Data Analysis for Site 2	0%	0%
7	Field Experiments at Site 3, Season 1	0%	0%
8	Field Experiments at Site 3, Season 2	0%	0%
9	Data Analysis for Site 3	0%	0%
10	Integrated Data Analysis for All Sites	0%	0%
11	Project management and reporting	13%	13%

SUMMARY OF SIGNIFICANT ACCOMPLISHMENTS

Data processing and analysis for the first round of sampling and toxicological assessment at Site 1 (Detroit) is continuing.

ACTUAL/ANTICIPATED PROBLEMS

No problems were encountered. We do not expect any problems for the next round of fieldwork, scheduled for February 11 - 23, 2006.

TECHNOLOGY TRANSFER ACTIVITIES

No technology transfer activities were performed in this quarter.